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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Krieg et al.
Serial No:	09/824,468
Confirmation No.:	9046
Filed:	April 2, 2001
For:	METHODS AND PRODUCTS FOR STIMULATING THE IMMUNE SYSTEM USING IMMUNOTHERAPEUTIC OLIGONUCLEOTIDES AND CYTOKINES
Examiner:	T. Gibbs
Art Unit:	1635

CERTIFICATE OF MAILING UNDER 37 C.F.R. 1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to, Mail Stop AF, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the 2nd day of October, 2003.

Pat RH Wall
PATRICK R.H. WALLER

MAIL STOP AF
Commissioner for Patents
P.O. BOX 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION OF DR. ARTHUR KRIEG UNDER 37 CFR §1.132

I, Dr. Arthur Krieg, declare as follows:

1. I make this Declaration in support of U.S. Serial No. 09/824,468 on which I am named as an inventor. I have reviewed the specification and pending claims of U.S. Serial No. 09/824,468. I have also reviewed the Office Action dated July 2, 2003.

2. I am the Senior Vice President for R&D and Chief Scientific Officer for Coley Pharmaceutical Group, and Professor of Medicine at the University of Iowa's Department of Internal Medicine (currently on leave). I have been performing research on CpG immunostimulatory nucleic acids for many years.

3. I have directed several experiments in the Coley research laboratories which examine the effects of combinations of CpG oligonucleotides and cytokines on the immune

response. The effect of these compounds on the immune response was assessed by measuring changes in the production of IFNα, IFNγ, and CD69. The experiments described in the following paragraphs confirm the teachings in the specification regarding the synergistic effect of CpG oligonucleotides in combination with specific cytokines on immune cells.

4. One set of experiments performed in the Coley research laboratories demonstrate the synergistic induction of IFNα in response to a combination of CpG oligonucleotide and cytokine IL-3. The experiments were performed by a PhD scientist, Ulrike Samulowitze, under my instruction and supervision. PBMC isolated from two different human donors were independently incubated with CpG oligonucleotide alone, IL-3 alone, or combinations of CpG oligonucleotide and IL-3 at different concentrations. The CpG oligonucleotide was ODN 2006 (5' TCGTCGTTTTGTCGTTTTGTCGTT 3') with a phosphorothioate backbone. The IL-3 was purchased from R&D Systems (Wiesbaden, Germany). The effect on the PBMC was assayed by measuring IFNα levels by ELISA. The results are attached hereto as Exhibit 1. The results demonstrate that a combination of CpG oligonucleotide and cytokine IL-3 has a synergistic effect on IFNα induction when compared to the effects of CpG oligonucleotide and cytokine IL-3 alone.

5. Another set of experiments performed in the Coley research laboratories demonstrate the synergistic induction of IFNγ in response to a combination of CpG oligonucleotide and cytokine IL-12. The experiments were performed by a PhD scientist, Ulrike Samulowitze, under my instruction and supervision. PBMC isolated from three different human donors were independently incubated with CpG oligonucleotide alone, IL-12 alone, or combinations of CpG oligonucleotide and IL-12 at different concentrations. Several CpG oligonucleotides were tested: ODN 2006 described above; ODN 2395 (5' TCGTCGTTTTCGGCGCGCGCCG 3') with a phosphorothioate backbone; and ODN 1982 (5' TCCAGGACTTCTCTCAGGTT 3') with a phosphorothioate backbone. The IL-12 was purchased from R&D Systems (Wiesbaden, Germany). The effect on the PBMC was assayed by measuring IFNγ levels by ELISA. The results are attached hereto as Exhibit 2. The results demonstrate that a combination of CpG oligonucleotide and cytokine IL-12 has a

synergistic effect on IFNγ induction when compared to the effects of CpG oligonucleotide and cytokine IL-12 alone.

6. Another set of experiments performed in the Coley research laboratories demonstrate the synergistic induction of CD69 in response to a combination of CpG oligonucleotide and cytokine IL-12. The experiments were performed by a PhD scientist, Ulrike Samulowitze, under my instruction and supervision. PBMC isolated from three different human donors were independently incubated with CpG oligonucleotide (ODN 2006, ODN 2395, or ODN 1982) alone, IL-12 alone, or combinations of CpG oligonucleotide and IL-12 at different concentrations. The expression of CD69 on human NK cells was determined using fluorochrome – conjugated CD69 antibodies (Becton Dickinson, Heidelberg, Germany) and flow cytometry on a FACSCalibur (Becton Dickinson). The results are attached hereto as Exhibit 3. The results demonstrate that a combination of CpG oligonucleotide and cytokine IL-12 has a synergistic effect on CD69 expression when compared to the effects of CpG oligonucleotide and cytokine IL-12 alone.

7. I, Dr. Arthur Krieg, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of this document and any patent which may issue from the above-identified patent application.

Date: 10/1/03 By: _____

Arthur Krieg